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Elucidating the contribution of microorganisms to the spontaneous fermentation and the quality of Ivorian cacao (*Theobroma cacao*) beans: The quality of Ivorian cacao (*Theobroma cacao*) beans

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Abstract

Cacao (*Theobroma cacao*) beans are among the most important ingredients in food and beverage industries. They are mainly produced in tropical and subtropical forests. Africa is the biggest producer of cacao bean providers in the world and the Ivory Coast remains the world leader with an estimated yearly production of 3 million tonnes. Cacao beans are used in many food items such as chocolate products, cocoa butter, confectionary products, iced drinks, cocoa powder, etc. The quality and organoleptic characteristics of these food products are strongly related to those of the cocoa beans obtained from different processing treatments. The pulps surrounding the cacao beans are rich in water, sugars, pectins, proteins, minerals, vitamins, citric acid, and phenolic compounds. Many different processing methods are utilized and fermentation is a crucial postharvest treatment having a great influence on the quality of cacao beans and their related products. Spontaneous fermentation is a common practice carried out by the cacao farmers in Ivory Coast. The microorganisms involved in this process are primarily the yeasts (anaerobic phase), which convert the pulps containing sugars into alcohol with a sporicidal temperature increase and then the lactic and acetic acid bacteria (aerobic phase) that produce lactic and acetic acids, respectively. The degradation of the substrates inside the cacao pulps results in the generation of aroma precursors and compounds. The goal of this review was to elucidate the factors affecting the spontaneous fermentation of Ivorian cacao beans and clarify the transformation of the raw material during fermentation.

Keywords: cacao beans; spontaneous fermentation; microorganisms; aroma precursors

Introduction

Seeds contained in the fruits of various plants have intensively been used by humankind for their health benefits in pharmaceutics, medicine, cosmetics domains, and as ingredient for flavoring agents, staple food, and drinks in the food and beverage industry. Processing techniques used in the food and related industries have a great effect on the product quality and consequently in consumers' preference and acceptance. Among the fruits containing seeds cultivated mainly in tropical forests, cacao beans are the most valuable and appreciated seeds by people around the globe for its unique taste, aroma, and mouthfeel. This is the reason for the tremendous consumption of its derived products.

Cacao tree (*Theobroma cacao*), classified in the Malvaceae family, is a plant grown in rainforest climate. It is the more intensive perennial crop cultivated in west-central Africa and south-west Asia among oil palm and rubber crops. The beans inside the fruits are utilized in the manufacturing of chocolate, cocoa butter, cocoa powder, and candy products (Guehi *et al*., 2010). Some countries have actively cultivated and adopted cacao trees as the main economic sources for their nations. The history of cacao dates back to 2000 BC in Mesoamerica where it was consumed as an unsweetened dark beverage by Mayans and Aztecs due to its beneficial and curative effects on human health discovered by the ancient people (Powis *et al*., 2011).

Cacao cultivation has greatly gained interest in some countries including Ivory Coast, Ghana, Nigeria, Cameroon, and Indonesia for centuries with its use as a key ingredient in food and cosmetic industries. The demand for cacao beans in world trade market has recently increased reaching about 4.5 million metric tons (Beg *et al*., 2017). Africa is the main supplier with approximately 73.3% of the global production while Ivory Coast is the biggest producer followed by Ghana (FAO, 2022). The three main cacao cultivars that have been largely recognized and cultivated in tropical and subtropical countries are "criollo trinitario," "forastero," and "nacional." The "criollo trinitario" cacao has a fine flavor and is exclusively produced for its extraordinary flavor characteristics and has 5–10% market share, while the "forastero" variety is designated as bulk cacao and is cultivated for its average flavor properties with its market share of about 90% (Afoakwa *et al*., 2009a), and it is usually used for the production of high-quality cocoa products (Rusconi and Conti, 2010). Furthermore, some cacao hybrids named as "nacional types" have newly emerged in tropical countries due to climate change, pests, and virus threats and they serve as better cultures to inhibit diseases with higher yields.

Ivory Coast, as mentioned above, is the world's leading exporter of cacao beans with an estimated annual production of 3 million tones covering around 40% of the global cacao production (FAO, 2022; Kone *et al*., 2020; Papalexandratou *et al*., 2011). Cacao tree is mostly planted in the southern, eastern, and western parts of the country providing significant income for the rural people in these localities. The main production areas of Ivorian cacao beans are La Nawa, Me and Marrhaoue. Among the perennial crops such as palm oil, rubber, coconut, and cashew cultivated in this country, cacao remains the backbone of the Ivorian economy. The main cacao cultivars adopted in the country are forastero, criollo, and nacional varieties, and among them, forastero is the most cultivated. The nacional variety is also called "Mercedes," which is a hybrid recently developed by the National Center of Agronomy Research (CNRA) of Ivory Coast.

Thanks to the recent progress of analytical tools in tandem with in vitro, in vivo, and clinical studies, cacao beans and its products have been reported to have crucial microelements, aroma compounds, and bioactive substances, mainly phenolic acids, flavan-3-ols, flavonols, anthocyanins, and psychopharmacological compounds, such as theobromine and caffeine (Bonetti *et al*., 2017; Kongor *et al*., 2016). These compounds have vital health benefits, such as antioxidative, anti-inflammatory, cardioprotective, antitumor, and antimicrobial properties, along with blood pressure reducing effects (Aydin *et al*., 2021; Misnawi *et al*., 2003). These elements (theobromine, caffeine, phenolics, etc.) have also been associated with unpleasant bitterness and astringency taste in unprocessed cacao beans, which makes them unacceptable for consumption. However, some efforts have been made to increase the pleasant compounds such as aroma concentration to appreciable levels and to reduce the unpleasant astringency and bitterness to a noticeable level so that they can be more palatable and highly accepted by consumers. These efforts are mostly carried out through fermentation process by cacao farmers and cacao processing industries (Afoakwa *et al*. 2011).

Postharvest processes, such as drying, roasting, alkalinization, and conching, significantly contribute to the flavor and quality improvement (Polat *et al*., 2021); fermentation remains the key operation affecting the quality of the product including some factors, such as fermentation techniques, conditions, microorganisms' populations, and the released aroma precursors. Fermentation is the primary and crucial postharvest process of the cacao beans for the mitigation of bitterness and astringency, the release of aroma compounds, and color development (Gálvez *et al*., 2007; Saltini *et al*., 2013). In general, cacao fermentation process is conducted spontaneously by driver microorganism populations present in the surrounding environment (Crafack *et al*., 2013). Cacao pulp or mucilage, which is the white part in raw beans, contains important amount of sugars, mainly glucose, fructose, sucrose, and pentose among other chemical compounds. During fermentation, the first stage is initiated by endogenic enzymes leading to the damage of tissues by the breakdown of proteins and carbohydrates into aroma precursors, such as sugars, free amino acids, and peptides, while the color changes from white to brown by the polyphenol oxidase enzyme activities (Camu *et al*., 2008; Elwers *et al*., 2009). Then, the yeasts naturally available in the fresh beans metabolize the fermentable sugars into alcohol and carbon dioxide (anaerobic) as the organic acids in aerobic conditions are bio-converted into lactic and acetic acid by bacteria. These processes promote the primary release of aroma compounds mainly alcohols, esters, aldehydes, ketones, and acids which strongly affect the fermentation time (Rodriguez-Campos *et al*., 2011). The growth and the survival of the broad microorganisms involved in fermentation depend on the environmental conditions (mainly oxygen and temperature), pH, time, and the composition of the cacao bean pulp used as substrate (Ouattara *et al*., 2017). Recent studies revealed that microorganisms engaged in cacao bean fermentation are heterogeneous and differ from region to region and from season to season (Daniel *et al*., 2009; Jespersen *et al*., 2005). For instance, in Ivory Coast, fungi and bacteria strains strongly vary from region to region (Koné *et al*., 2016; Ouattara *et al*., 2017).

The aim of this work was to reveal some details of the spontaneous fermentation process of Ivorian cacao beans and clarify the transformations of the raw material during fermentation with respect to some quality parameters of the cocoa products.

Cacao Bean Spontaneous Fermentation Setup

It was well established for centuries that the cacao fermentation aimed to remove the mucilaginous pulps surrounding the cacao beans by the action of the complex ubiquitous microorganisms to ease the continuity of the postharvest process. In recent years, instrumental analyses have clearly indicated that throughout fermentation process, cacao beans undergo a series of transformation inside and outside by inducing significant biochemical changes (Figure 1), which improve their quality and the formation of key precursors of a broad array of Maillard reaction that leads to the characteristic flavor, aroma, and color of end products (D'Souza *et al*., 2018; Villeneuve *et al*., 1989). Indeed, the pulp covering the cacao beans contain a large amount of water (80–90%), sugars (10–15%) mainly sucrose, citric acid (1–3%), pectin (1–1.5%), proteins (0.5–0.7%), minerals (8–10), and vitamin C, which represent a good substrate for microbial growth (Lefeber *et al*., 2011). Cacao bean fermentation is generally performed in a spontaneous and artisanal way on cacao farms, and can differ from country to country and from farm to farm (Lima *et al*., 2011). The fermentation technique can be classified into five groups according to the place it happens, such as fermentation in heaps (Ivory Coast, Ghana); trays (Ivory Coast, Ghana); barrels or baskets (Ivory Coast); boxes—plastic or banana leaf boxes (Ivory Coast, Ghana), wooden boxes (Ivory Coast, Ghana, Brazil, Malaysia); and platforms (Ecuador).

The cacao bean fermentation is mainly carried out in boxes and last 2–10 days with interval rotation of the beans depending on the farms or cocoa-producing regions (Thompson *et al*., 2012). The duration considerably influences the product quality depending on the cacao cultivar, growing region, harvest season, preharvest practices, and fermentation method. For instance, the forastero cultivar with its pronounced flavor needs 5 to 8 days to ensure full flavor, aroma, and color precursor development while the criollo variety requires a shorter period (Wood and Lass, 2008). A well-performed fermentation is a precondition to obtain a good-quality cocoa product but if it is done improperly, the release of specific flavoring constituents during fermentation will be lower reducing the quality (Crafack *et al*., 2013).

In Ivory Coast, the fermentation is commonly carried out spontaneously in banana leaf boxes or heaps in small plantations or in wooden or plastic boxes on bigger farms operated as bioreactors (Guehi *et al*., 2007). The prevalent duration is generally between 4 and 6 days with a rotational bean turning up twice per day to obtain a uniform aeration. The wooden, banana , or plastic boxes with different sizes from 20 to 2000 kg of beans have holes to evacuate the juice resulted from the liquefaction or degradation of the mucilaginous pulps (Jinap *et al*., 1995). Some unpleasant flavors may be generated by the fungal and *Bacillus* species after an extended fermentation time. The fermentation duration is entirely related to weather conditions and available nutrients in the pulps.

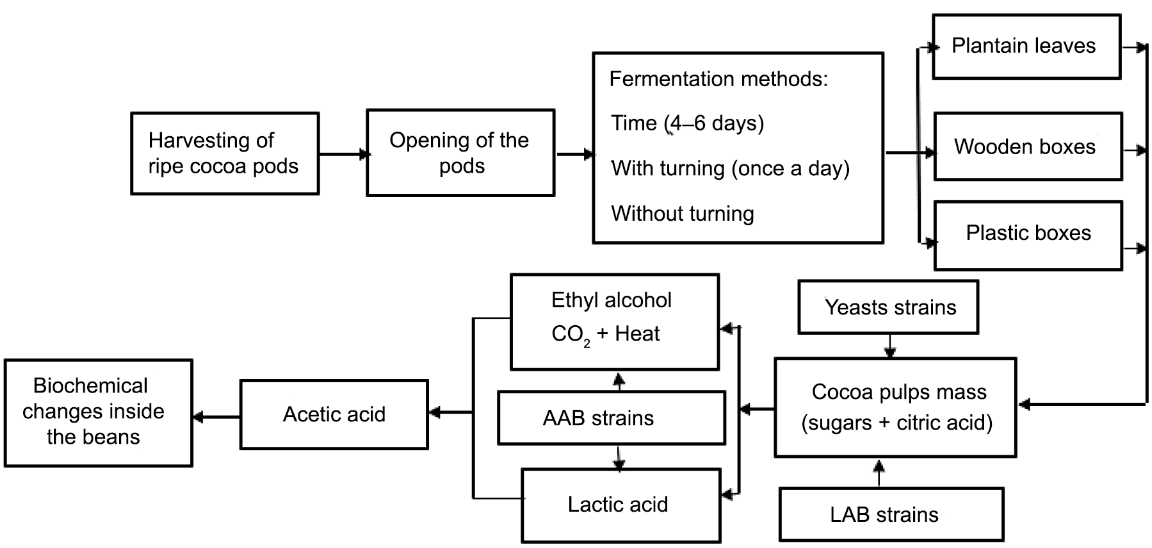

Figure 1. Spontaneous cacao bean fermentation methods performed in Ivory Coast and induced microbiological and biochemical changes. LAB (Lactic acid bacteria), AAB (Acetic acid bacteria).

The presence of microorganisms on the cacao pod surface, on the nonsterile utensils, and around the surrounding fermentation environment is a good inoculation source to decompose the mucilaginous pulp of the beans used as substrate (Guehi *et al*., 2010).

Microorganism Populations Involved During Cacao Bean Fermentation Process

The spontaneous fermentation of fresh cacao beans is induced by a wide range of microbial strains sourced (Table 1) from the surface of cacao pods, farmers, materials used in the harvest and postharvest process, and utensils such as wooden tools, plantain leaves, and plastic boxes used in fermentation. Among the overall microbial population, only yeasts, lactic acid, and acetic acid strains lead to fermentation process (Camu *et al*., 2008). In this stage of bioconversion, the sugars and associated polysaccharides are mainly digested by yeasts in anaerobic condition. During this stage, ethanol, organic acid, and free amino acids are produced inducing a reduction of the pH level. The endogenous enzymes (invertases, glucosidases, proteases, and polyphenol oxidases) in cacao

Table 1. Microorganisms identified during cacao fermentation in different regions of Ivory Coast.

Regions	Microbial species	References
	Yeasts	
Abidjan	*S. cerevisiae*, *C. tropicalis*, *P. kudriavzevii*, *P. galeiforms*, *G. geotrichum*, *W. anomalus*	Koné *et al.* (2016)
Akoupe	*G. geotrichum*, *C. tropicalis*, *P. kudriavzevii*, *S. cerevisiae*, *P. galeiformis*, *W. anomalus*, *P. manshurica*, *C. ethanolica*, *P. deserticola*, *H. Opuntiae*	Koné *et al.* (2016), Hamdouche *et al.* (2019)
Agneby Tiassa	*C. nitrativorans*, *P. Kudriavzevii*, *C. tropicalis*, *C. intermedia*, *C. nocodendra*, *H. uvarum*	Samagaci *et al.* (2014)
Nawa	*S. cerevisiae*, *H. uvarum*, *P. fermantans*, *P. kudriavzevii*	Ouattara and Niamké (2021)
	Lactic acid bacteria (LAB)	
San-Pedro	*L. plantarum*, *Lc. mesenteroides*	Ouattara and Niamké (2021)
Loh-Djiboua	*L. curieae*, *Lc. mesenteroides*, *W. Paramesenteroides*, *L. plantarum*, *E. feacium*	Ouattara and Niamké (2021), Ouattara *et al.* (2017)
Sud-Comoe	*L. plantarum*, *Lc. mesenteroides*, *W. Paramesenteroides*	Ouattara *et al.* (2017)
Nawa	*L. plantarum*, *Lc. mesenteroides*, *W. paramesenteroides*, *L. curieae*	Ouattara *et al.* (2017), Ouattara and Niamké (2021)
Akoupe	*L. fermentum*, *L. curvatus*, *E. casseliflavus*	Hamdouche *et al.* (2019)
Agneby-Tiassa	*L. plantarum*, *Lc. mesenteroides*, *W. ciberia*	Ouattara *et al.* (2017)
Guemon	*L. plantarum*, *Lc. mesenteroides*, *L. casei*, *L. curieae*	Ouattara *et al.* (2017)
Indenie-Djuablin	*L. plantarum*, *Lc. meseneroides*, *L. casei*, *L. curieae*	Ouattara *et al.* (2017)
	Acetic acid bacteria (AAB)	
Akoupe	*A. pasteurianus*	Hamdouche *et al.* (2019)
Agneby-Tiassa	*A. pasteurianus*, *A. tropicalis*, *A. okinawensis*	Soumahoro *et al.* (2020)
Guemon	*A. pasteurianus*, *A. tropicalis*, *A. okinawensis*	Soumahoro *et al.* (2020), Ouattara and Niamké (2021)
Indenie-Djuablin	*A. pasteurianus*, *A. tropicalis*, *A. okinawensis*, *A. malorum*, *G. oxydans*	Soumahoro *et al.* (2020)
Nawa	*A. pasteurianus*, *A. tropicalis*, *A. okinawensis*	Soumahoro *et al.* (2020), Ouattara and Niamké (2021)
Sud-Comoe	*A. pasteurianus*, *A. ghanensis*, *A. tropicalis*, *A. okinawensis*	Soumahoro *et al.* (2020)
Loh Djiboua	*A. pasteurianus*, *A. tropicalis*, *A. okinawensis*	Soumahoro *et al.* (2020), Ouattara and Niamké (2021)
Goh	*A. okinawensis*, *A. ghanensis*, *A. tropicalis*, *G. oxydans*, *A. pasteurianus*	Ouattara and Niamké (2021)
	Bacillus species	
Akoupe	*B. megaterium*	Hamdouche *et al.* (2019)
Abidjan	*B. cereus*, *B. thurengensis*, *B. Subtilis*, *B. fusimorfis*, *B. sphearicus*, *B. pumilus*	Ouattara *et al.* (2010)

pulps play a remarkable role during fermentation and they ensure the characteristic cocoa flavor and color (Afoakwa *et al.*, 2009b). In addition, pectinolytic enzymes present in the pulps degrade the pectin into aroma precursors such as fatty acids, alcohols, and fatty esters (Illeghems *et al.*, 2012). After 1–2 days, the alcohol concentration and temperature drastically increase, which consequently inhibit the yeast population activity. Then, the medium containing residual sugars, citric acid, and oxygen becomes suitable for the growth of lactic acid bacteria (LAB) by bio-converting these substrates into lactic acid, mannitol, and low concentration of acetic acid. The increased amount of oxygen in the medium consisting mainly of lactic acid and high quantity of ethanol used as substrates by acetic acid bacteria (AAB) are converted into acetic acid, carbon dioxide, and water. All the compounds liberated at the different stages of fermentation are very important in the development of aroma complexes.

Yeast species

Diverse populations of yeasts found in cacao bean fermentation have been reported in cocoa-producing countries. Recent studies on yeast diversity have shown an impressive presence of different strains belonging to the genera *Candida, Kluyveromyces, Hansenula, Kazachstania, Pichia, Meyerozyma, Hanseniaspora, Rhodotorula, Saccharomyces, Saccharomycopsis, Cryptococcus, Wickerhamomyces,* and *Schizosasacchromyces* (Ardhana and Fleet, 2003; Mota-Gutierrez *et al.*, 2018). These yeast strains strongly differ from region to region, from season to season, and even from plantation to plantation as well as the fermentation practices performed. For example, very distinct yeast strains have been identified to be the most dominant species during cacao bean fermentation in Brazil (*P. kluyveri, S. cerevisiae, H. ovarum,* and *I. orientalis*), Cuba (*S. cerevisiae, C. magnoliae, P. kluyveri, C. tropicalis,* and *P. occidentalis*), Ghana (*S. chevalieri, C. krusei, T. candida, S. cerevisiae,* and *H. ovarum*), and Mexico (*P. kundriavzevii, S. cerevisiae, S. crataegensis,* and *H. guilliemondii*), while yeast species of *S. cerevisiae, C. tropicalis, P. kundriavzevii, P. kluyveri,* and *P. galeiforms* have been determined to be the most abundant in different cacao-producing regions of Ivory Coast (Daniel *et al.*, 2009; Koné *et al.*, 2016; Miguel *et al.*, 2017; Pereira *et al.*, 2012). *S. cerevisiae* was identified as the most prevalent yeast leading to the production of ethanol in the early stage of the fermentation (Schwan, 1998).

As shown in Table 1, several microorganisms implied in Ivorian cacao beans fermentation have been well established. For instance, Koné *et al.* (2016) indicated that 10 strains of yeasts in cacao fermentation in different regions of Ivory Coast among which *S. cerevisiae, G. geotrichum, W. anomalus*, and *P. kudriavzevii* intensively contributed to the development of characteristic aroma components. In another study in Ivory Coast by Hamdouche *et al.* (2015), nine yeast species including *H. opuntiae*, *C. insectorum*, *P. kundriavzevii, P. klyvera, P. fermentans, P. galeiformis*, *P. manshurica*, *P. sporocuriosa*, and *I. hanoiensis* were identified at different times of fermentation and these yeast species varied heterogeneously throughout the fermentation. The fermentation technique greatly influenced the yeast population as indicated by Koné *et al.* (2016) using banana leaves (*G. geotrichum, C. tropicalis, P. kudriavzevii,* and *P. galeiforms*), wooden boxes (*G. geotrichum, P. kudriavzevii, S. cerevisiae,* and *P. galeiforms*), and plastic boxes (*C. tropicalis, P. kudriavzevii,* and *W. anomalus)* during cacao bean fermentation. In addition, *P. kudriavzevii* and *Hanseniaspora sp.* were abundantly determined at the first stage of the Ivorian cacao bean fermentation using heaps (Papalexandratou *et al.*, 2009).

Lactic acid bacteria species

LAB strains gradually increase after the decline of yeast population in the medium due to the large reduction of liquid by drainage of the pulp, the elevated temperature and oxygen levels. In a certain way, the yeast strains promote the growth of LAB species. Different LAB strains involved in cacao bean fermentation have been clearly studied using classical and advanced microbiological tools in many cacao-growing countries. The order of LAB population fermenting the cacao beans is similar worldwide. However, these LAB species have been reported to differ from country to country, weather to weather, farm to farm, and based on the fermentation methods employed. Different strains of the genera *Lactobacillus, Leuconostoc, Pediococcus, Weisella,* and *Lactococcus* reportedly control the cacao fermentation process. Many works conducted in different regions commonly identified the *Lactobacillus plantarum* and *Lactobacillus fermentum* as the prevalent heterofermentative LAB species in cacao bean fermentation. Studies highlighted that *L. cellobiosus* and *L. plantarum* were mainly identified in Indonesia (Ardhana and Fleet, 2003); *L. fermentum, L. plantarum*, *L. brevi,* and *Leuconostoc* (*Lc*). *mesentroides* were predominant in Malaysia (Meersman *et al.*, 2013); *L. plantarum*, *L. fermentum*, *L. brevi, L. mali,* and *W. ghanensis* were prevalent in Ghana (De Bruyne *et al.*, 2010); *L. plantarum, L. fermentum, L. brevi,* and *P. acidilactici* were dominant in Nigeria (Kostinek *et al.*, 2008), while *L. plantarum, L. fermentum, L. casei, L. lactis, L. acidophilus, L. brevis,* and *Lc. lactis* were mostly identified in Brazil (da Veiga Moreira *et al.*, 2013; de Melo Pereira *et al.*, 2013).

Regarding prevalence of the LAB species in Ivory Coast, *L. plantarum, Lc. mesentroides, L. curieae, L. casei, W. paramesenteroide,* and *W. cibaria* were mainly detected among which *L. plantarum* and *Lc. mesentroides* were the most abundant in cacao fermentation from six regions (Ouattara *et al.*, 2017). In a study conducted by Hamdouche *et al.* (2015), two LAB species, namely, *L. plantarum* and *L. fermentum*, were detected with the abundance of *L. plantarum* during the Ivorian cacao bean fermentation. The presence of *L. curvatus* and *L. fermentum* was also indicated in another study with different factors (time and beans turning) affecting the fermentation process (Hamdouche *et al.*, 2019). In a similar study by Papalexandratou *et al.* (2011) on the variable fermentation time, *L. fermentum* was prevalently detected after 30 h and *Lc. pseudomesenteroide* was abundantly present after 54 h of fermentation. *L. plantarum* was present in all cacao bean fermentation in different regions, cocoa farms, and it was even detected in all fermentation techniques applied in Ivory Coast.

Acetic acid bacteria species

The drastic increase of oxygen, temperature, and metabolic products (ethanol, lactic acid, and mannitol) during the first two stages of cacao bean fermentation with the significant diminution of yeast and bacteria population favor the growth of AAB species. Ethanol, and in some instances lactic acid and mannitol are converted into acetic acid by AAB. This last one deeply penetrates into the cotyledon increasing the interior acidity of the beans (Ardhana and Fleet, 2003; Soumahoro *et al.*, 2020). Recent investigations on this last step of fermentation indicate a plethora of heterogeneous AAB species belonging to the genera *Acetobacter* and *Gluconobacter. Acetobacter* species are the most dominant in cacao bean fermentation worldwide (Camu *et al.*, 2008; Papalexandratou *et al.*, 2011; Soumahoro *et al.*, 2020). Among them, *A. pasteurianus* was identified as the most prevalent in different cacao bean– producing regions (Gálvez *et al.*, 2007). Geographical studies on AAB strains fermentation emphasized that *A. pasteurianus* was the only one determined in Indonesia and Malaysia (Ardhana and Fleet, 2003); *A. pasteurianus, A. senegalensis*, and *A. tropicalis* were abundant in Ghana (Camu *et al.*, 2008), while *A. aceti, A. pasteurianus*, *A. ghanensis, A. senegalensis, A. cerevisiae,* and *G. saccharivorans* were reported in various regions of Brazil (Illeghems *et al.*, 2012).

Recent studies conducted by some Ivorian researchers showed many species of *Acetobacter* and lower number of *Gluconobacter* species in the fermentation of Ivorian cacao beans. These LAB species were composed of *A. pasteurianus, A. tropicalis, A. okinawensis, A. malorum, A. ghanensis*, and *G. oxydans* (Soumahoro *et al.* 2020). Hamdouche *et al.* (2019) studied some factors (beans turning time) influencing the fermentation process and determined *A. pasteurianus* as the most dominant AAB species in Akoupe (Ivory Coast). Moreover, a 6-day fermentation process using wooden boxes revealed the presence of *Acetobacter* (*A*). *nitrogenifigens, A. lovaniensis, A. cerevisiae*, *A. pasteurianus*, and *Gluconoacetobacter xylinum* in Ivorian cacao bean fermentation (Hamdouche *et al.*, 2015). Ouattara and Niamké (2021) identified seven AAB species isolated in 12 cacao-producing regions of Ivory Coast including *A. pasteurianus, A. okinawensis, A. ghanensis, A. tropicalis, A. senegalensis, A. malorum*, and *G. oxydans*. In Ivory Coast, *A. pasteurianus* was the most detected LAB species in all cacao bean fermentation methods applied in the country.

Bacillus *species*

These groups of bacteria are classified into spore-forming bacteria and significantly appear in the later stages of fermentation with the increase of aeration, pH value (3.5 to 5.0), and elevated temperature to about 45–50°C (Binh *et al.*, 2017). Some isolated *Bacillus* species have the capacity to bio-convert all metabolic products (ethanol, lactic acid, mannitol, and acetic acid) into unwanted chemical compounds. Indeed, they have been reported to contribute to the generation of off-flavor components in fermented cacao beans. The *Bacillus* species mostly identified in cacao beans are *B. brevis*, *B. cereus*, *B. coagulans*, *B. firmus, B. licheniformis*, and *B. subtilis* in Brazil (Schwan, 1998); *B. cereus, B. coagulans, B. licheniformis*, and *B. subtilis* in Trinidad (Ostovar and Keeney, 1973); *B. licheniformis* and *B. subtilis* in Ghana and Malaysia, while *B. cereus* and *B. subtilis* are the most dominant species in Ivory Coast (Ouattara *et al.*, 2010). Although variations were observed in the distribution of *Bacillus* species according to different regions or countries, some species were commonly reported.

The recent mapping of main microbial strains involved in Ivorian cacao bean fermentation showed the presence of *B. megaterium, B. subtilis, B. fusiformis, B. thuringensis*, and *B. pumilus* in different cacao-producing regions, which may vary from season to season (Hamdouche *et al.*, 2015).

Factors Affecting the Fermentation Process

The populations of microorganisms involved in cacao bean fermentation undergo several disruptions due to a number of factors, which sometimes contribute to an incomplete fermentation process. The chronological activities of the microorganisms during fermentation and the factors that influence their dynamism remain similar in all cocoa-producing regions or countries. These microbial activities are reported to be considerably affected by internal and external factors such as pH, temperature, time, climate, season, turning of beans, fermenter batch size, oxygen level, amount of beans, pulps preconditioning, methods used, age of pods, the successive metabolic products, etc. (Afoakwa *et al.*, 2011). The variation of the microorganisms at the different stages of fermentation is due to the changes in oxygen, temperature, ethanol, pH, or acidity. Among them, oxygen, temperature, pH, and metabolic products have strong effects on the fermentation (Lima *et al.*, 2011).

Aeration

At the onset of the fermentation, the oxygen concentration inside the bean mass is very low due to the firm heap of the bean mass, which enables a rapid growth of yeasts species. At this stage, the activities of the bacteria species are extremely low (Lima *et al.*, 2011). The use of the substrates mainly sugars by the yeasts creates a disruption of the bean tissue structure resulting in ethanol production. Turning of beans in short intervals associated with the embrittlement of bean pulp tissues promotes a strong ingress of oxygen. The yeast populations significantly decrease after some time (24–72 h) due to the high level of oxygen and ethanol. Although some yeast species have been identified at the end of the fermentation, most of those contributing to the development of aroma are inhibited at 2 or 4 days of fermentation. The turning up of cacao beans allows air to enter inside the bean mass, which boosts the growth of LAB and continuous aeration promotes the expansion of AAB and *Bacillus* species at the last stage of the fermentation (Camu *et al.*, 2008). Some *Bacillus* species are known to contribute to the production of off-flavor due to high level of oxygen during the cacao bean fermentation process. The oxygen distribution during the fermentation depends on the method of fermentation used.

Temperature level

The spontaneous fermentation of fresh cacao bean generally starts at ambient temperature. Yeasts species thrive well at this range of temperature and reach their maximum activities, resulting in the generation of first metabolic products, mainly ethanol (De Vuyst and Weckx, 2016). With the higher concentration of ethanol, lactic acid, and aeration, AABs transform the first-stage metabolic products (ethanol, lactic acid, and mannitol) into acetic acid. This bioconversion is an exothermic reaction resulting in the rise of the temperature (55–60°C) within the beans during the first 2–3 days of the fermentation and then starts to decline at the end of the fermentation (Camu *et al.*, 2008; Lima *et al.*, 2011). Papalexandratou *et al.* (2011) elucidated the consequence of the variation of temperature with different fermentation methods on microbial species and their metabolites in selected Ivorian cacao beans. They indicated a progressive increase of temperature initially from 23–24°C to 42–48°C after 96–117 h of fermentation in heap and box mediums, respectively.

pH level

The pH of cacao pulp is a crucial factor that strongly influences the behaviors of microbial species during cacao mass bioconversion. It was highlighted in previous studies that the initial pH of fresh cacao pulp is around 3.5–3.7, and it gradually rises to 4.5 within 24–48 h and to 6.5 after 168 h of fermentation (Lefeber *et al.*, 2010). The cacao pulp naturally contains low amount of citric acid (around 1%) which strongly impacts the pH value. The yeasts responsible for the production of ethanol are inhibited at the first stage by the degradation effect of citric acid, which gradually rises the pH value and the medium becomes more suitable for the development of LAB (Schwan, 1998). In the last stage of the fermentation, when the pH is between 4 and 5 causing an intense respiration of yeasts and LAB, the fermentation slows down making the medium more favorable for AAB to thrive on ethanol and lactic acid. The rapid increase of the pH in the early stage of the fermentation is detrimental for the growth of yeast and LAB, which subsequently decreases the fermentation rate and negatively impacts the quality and release of cacao aroma precursors. In addition, successful cacao bean fermentation is characterized by the gradual rise in pH value up to 5.0 and when the pH is above 5.0 and reaches up to 8.0, bacteria such as some *Bacillus* species lead the fermentation producing off-flavor (Samagaci *et al.*, 2014). It is indicated that fermentation with a pH of about 5.0 yields good-quality cocoa beans with specific aroma; however, when the pH is under 5.0, the beans have a poor quality with acidic taste (Janek *et al.*, 2016).

Metabolic products

Ethanol and acids are the main metabolic products generated during cacao bean fermentation. These products seem to greatly influence the microbial species involved in this bioconversion and the cell death due to the diffusion of these metabolites inside the beans (Schwan, 1998). For instance, high concentration of ethanol strongly inhibits the activities of some yeast species in the first hours of the fermentation. In addition, an accretion of acids limits the actions of the bacteria and influences the aroma, flavor, and quality of the cocoa beans (Lefeber *et al.*, 2011).

Extreme acidification during fermentation also results in lowered quality. An early decomposition of citric acid in the pulp mass by some yeast or LAB strains noticeably change the pH of the medium, which influence the yeast population growth, resulting in lower ethanol production affecting the conversion to acetic acid.

The Release of Aroma Precursors and Aroma Compounds during Fermentation

As can be observed in Figure 2, proteins in cacao pulp mass and within the beans are metabolized into peptides and free amino acids, namely, internal precursors (tyrosine, alanine, and leucine) and external precursor (phenylalanine) by proteolysis reaction induced by carboxypeptidase and certain *Bacillus* and yeasts strains. These are the main aroma precursors and they significantly contribute to the aroma of the fermented cocoa and its related products (Beckett, 2009; Janek *et al.*, 2016; Kratzer *et al.*, 2009). The acetic acid and the residual lactic acid with the collaborative action of endogenous enzymes such as aspartic endo-protease and carboxypeptidase activate the production of aroma precursors. The fructose and glucose obtained from sucrose by invertase present in yeasts forms the precursors of aroma components during the first stage of the fermentation. The sucrose molecules inside the beans are also converted into glucose and fructose by the diffusion of ethanol, acetic acid, and lactic acid together with the combination of heat (de Melo Pereira *et al.*, 2013). Fructose and glucose actively develop the aroma substances by their reaction of amino acids and peptides at the second and third steps of cacao beans postharvest process (Afoakwa *et al.*, 2009a). All of the aroma precursors produced by the microbial action during fermentation are almost similar but vary significantly in their amount, which could be due to fermentation type, practices, weather, preharvest conditions, and geographical factors.

Aroma compounds greatly affect the cocoa bean quality and derived products and, thus, their acceptance by the consumers. Aroma components generated at the first stage of the cacao postharvest process, i.e., the fermentation, have been studied in recent years in cacao-producing countries. As indicated in Table 2, aldehydes, alcohols, acids, esters, ketones, and pyrazines produced by yeasts, LAB, AAB, and *Bacillus* species are the main aroma groups found in fermented Ivorian cacao beans (Hamdouche *et al.*, 2019; Koné *et al.*, 2016). The liberation and concentration of the aroma compounds during fermentation are time-dependent. These compounds are derived from amino acids during biochemical reaction pathways, which contribute to the fruity, floral, herbal, grassy, chocolate/nutty, and buttery odors of cocoa bean products.

Recent studies conducted in Ivory Coast showed important amounts of aroma compounds in fermented Ivorian cocoa beans. For instance, Hamdouche *et al.* (2019)

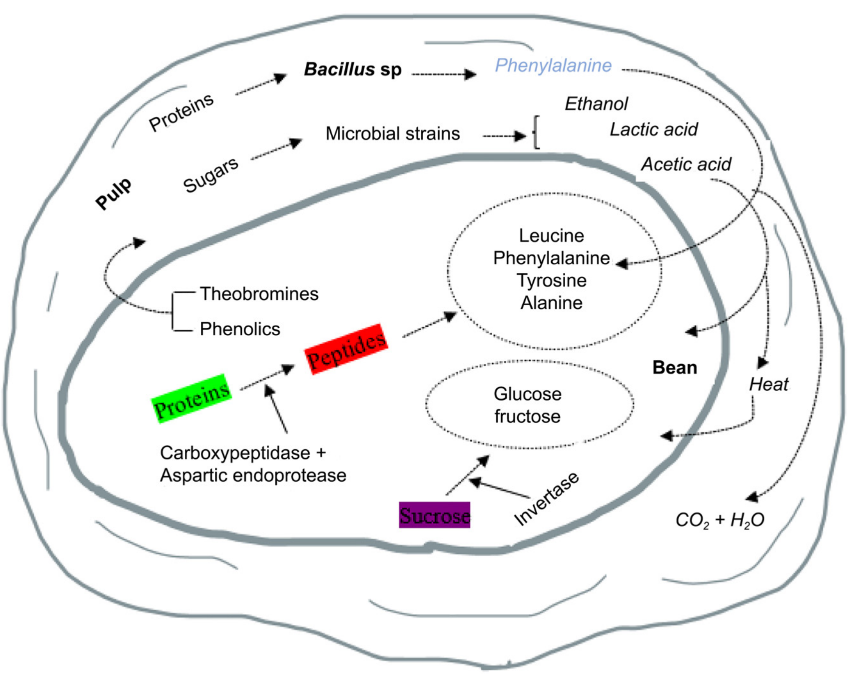

Figure 2. The release of aroma precursors during cacao bean fermentation by microbial strains and their related enzyme activities.

Table 2. Volatile compounds in fermented Ivorian cacao beans from different regions.

Aroma compounds	Odor descriptors	Microbial strains involved
Aldehyde	**Fruity and floral**	
Isobutanal	Alcoholic	ND
3-Methyl butanal	Chocolate	ND
2-Methyl butanal	Chocolate	ND
Benzaldehyde	Sweet, almond	*G. geotrichum, A. pasteurianus*
Ketones	**Fruity**	
2-Pentanone	Fermented, ripe banana	*A. pasteurianus*, *L. fermentum, P. kurdriavzevii*
2-Heptanone	Fruity, coconut, floral	ND
2-Nonanone	Herbal, fresh sweet,	*P. manshurica, P. kurdriavzevii*
Acetophenone	Sweet, cherry	ND
Acetoin	Butter-like, creamy	*A. pasteurianus*, *L. fermentum*
Esters	**Fruity, sweet**	
Ethyl acetate	Pineapple, fruity, sweet	*G. geotrichum, C. tropicalis, A. pasteurianus*
Methyl isovelerate	Green, fruity	*G. geotrichum*
Isobutyl acetate	Fruity	*G. geotrichum, C. tropicalis, S. cerevisiae*
Isoamylpropanoate	Fresh fruity, sweet	S. cerevisiae
Isoamyl acetate	Banana, fruity	*G. geotrichum, C. tropicalis*
Phenylethyl acetate	Floral, honey,	*P. kurdriavzevii*
Isoamyl valerate	Fruity	*G. geotrichum, C. tropicalis*
Ethyl-2-butenoate	Fruity, pineapple	*G. geotrichum*
Ethyl decanoate	Pineapple-like	*P. kurdriavzevii*
Butyl acetate	Fruity	*L. fermentum, P. kurdriavzevii*
Isobutyl acetate	Sweet, fruity	*A. pasteurianus*
Alcohols	**Fermented, fruity, alcoholic**	
Ethanol	Alcoholic	*P. manshurica, P. kurdriavzevii*
2-Pentanol	Fermented, musty	*A. pasteurianus*, *L. fermentum, P. kurdriavzevii*
Isopentanol	Wine-like	*L. fermentum, P. manshurica, P. kurdriavzevii*
Isobutanol	Wine	*S. cerevisiae, G. geotrichum, C. tropicalis*
2-Heptanol	Banana-like, fruity	*ND*
2,3-Butandiol	Alcoholic	*S. cerevisiae*
2-Phenylethanol	Floral	*P. manshurica, P. kurdriavzevii*
3-Methylbutanol	Meaty	*ND*
Isoamyl alcohol	Malty, bitter, chocolate	*ND*
2-Heptanol	Fresh Grass, floral, sweet	*ND*
2-Nonanol	Fruity, floral	*ND*
Acids	**Rancid, sweet, Buttery**	
Acetic acid	Vinegary, sour	*A. pasteurianus*, *L. fermentum, P. Kurdriavzevii*
Isobutyric acid	Butter, rancid	*A. pasteurianus*
Isovaleric acid	Sweet, acid, rancid	*A. pasteurianus*, *L. fermentum, P. manshurica*
Octanoic acid	Rancid, oily	
3-Methylbutanoic acid	Sweet, rancid	*ND*

Continued

Table 2. Continued.

Aroma compounds	Odor descriptors	Microbial strains involved
Others		
Tetramethylpyrazine	Roasted nut,	*ND*
3-Phenylfuran	Cocoa, minty, green	*ND*
2-Methoxy-phenol	Phenolic-like, woody	*ND*

Sources: Hamdouche *et al.* (2019), Koné *et al.* (2016), ND: Not detected.

identified 26 compounds and the most dominant ones were aldehydes (benzaldehyde, 3-methylbutanal), ketones (acetoin), esters (ethyl acetate), alcohols (2-phenylethanol, 2,3-butanediol), and acids (acetic acid) in all fermentation types. The authors pointed out a strong relationship between microbial populations and volatile compounds released. In another study, a total of 33 aroma components from four main classes, namely, esters, alcohols, ketones, and acids were detected in fermented Ivorian cocoa beans in Abidjan which were mainly produced by yeast strains such as *S. cerevisiae, G. geotrichum, P. kudriavzevii*, and *W. anomalus* (Koné *et al.*, 2016).

Conclusions

The article aimed to reveal some details of the spontaneous fermentation process of Ivorian cacao beans and clarify the transformations of the raw material during fermentation with respect to some quality parameters of the cocoa products.

Previous investigations showed that fermentation of cacao beans is a crucial and indispensable postharvest process for the mitigation of the bitterness and astringency compounds of cacao beans. Although controlled fermentation using starter culture is emerging in some cacao bean–producing countries, spontaneous fermentation remains the most performed in many countries including Ivory Coast. Aroma precursors and aroma compounds released during fermentation are the results of the synergistic work of diverse populations of microorganisms present on the cacao pod surface, unsterilized tools, and the surrounding environment. Several factors such as aeration, pH, and temperature also influence the fermentation process significantly. Various microbial species are involved in the fermentation of Ivorian cacao beans and this process significantly differs from region to region. These microbiota ecosystems together with their enzymes have crucial impacts on the production of aroma precursors, flavors, and color of the fermented and its derived cocoa products.

It has been clearly shown in this present review that microorganisms including yeasts (*S. cerevisiae, C. tropicalis, P. kudriavzevii,* and *G. geotrichum*), LAB (*L. plantarum, Lc. mesenteroides,* and *L. curieae*), AAB (*A. pasteurianus, A. tropicalis,* and *A. okinawensis*), and *Bacillus* species (*B. cereus*, *B. megaterium*) are the principal microbials mostly involved in Ivorian cacao bean fermentation inducing biochemical changes in and outside of the beans. The parameters, namely, temperature (25–60°C), pH (3–5), and aeration play important roles during cacao bean fermentation. In addition, fermentation induces the reduction of sugars, bitter and astringent tasting compounds, while increasing acetic acid, aroma, and aroma precursor compounds in cacao beans. It is also stated that the good quality of cacao beans is obtained at pH 5; when the pH is under or above 5 cacao beans are in poor quality due to acidic taste and off-flavor production.

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